Assessment of iron status

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Purpose of review
Iron is an essential trace element in human health that can be harmful at abnormal levels such as iron overload or deficiency. Measured iron status in the body can depend on health outcomes experienced by the individual and this can complicate its accurate assessment. This review will highlight recent research on iron assessment in the literature.

Recent findings
Research on iron assessment within the past 18 months included some common themes spanning new methods and biomarkers, as well as existing problems in assessing iron deficiency and overload. Heterogeneity in associations between inflammation and iron levels are reflected across different inflammatory biomarkers. New methods relevant to low- and high-resource settings may improve assessment in tissues with iron deficiency and overload. Consensus papers outlined best practices when using MRI to assess iron status. Outside of newer methods, traditional serum markers are the subject of a call for updated guidance when assessing iron status.

Summary
Research continues on the topic of iron assessment, underlying its complex metabolism in the body and resulting challenges in assessment. Current literature underscores progress to make iron assessment more accessible, improve existing methods, and update current assessment methods so they correspond with recent research to improve human health.

Keywords
anemia, biomarkers, healthcare, iron, iron overload, iron-deficiency, outcome assessment

INTRODUCTION

Iron is an essential trace element necessary for human health. However, at abnormal levels, iron can have adverse health outcomes in the body arising from mechanisms including nutritional intake, genetics, and absorption. A large proportion of the world-wide population has iron levels requiring assessment and intervention. For example, globally, one in four women are estimated to have iron deficiency [1]. Assessment of iron requires an understanding of its homeostasis in the body, a complex process reflected by the ongoing search for meaningful, accurate and noninvasive measures of assessment. Given its complex role in human health, iron status assessment relates to different target populations and topics such as the prevalence of iron deficiency and overload as well as specific patient populations for whom iron levels require intervention. As noted in the following literature review, assessment of iron status using common serum indicators may not be ideal [2] and may not represent the true iron status of an individual thus impairing efforts at treatment and intervention. Highlighting advances in methods, information, biomarkers relating to iron assessment can inform future research on iron as both an outcome and exposure. The aim of this paper is to summarize recent studies that have a primary focus on iron assessment, categorized by most frequent topics in the literature from the past 18 months.

FACTORS INFLUENCING THE ASSESSMENT OF IRON STATUS

There are many factors associated with iron status, which can be used to better understand the limitations of its assessment and potential roles for confounders when evaluating iron status as an exposure.

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KEY POINTS

- Screening methods such as MRIs are advancing the understanding of tissue-specific iron metabolism, which can better inform interventions and noninvasive research.
- Gaps in the assessment of iron deficiency still exist for the general population and among those with inflammatory conditions with recent publications advocating for change.
- Some research focuses on novel approaches to iron assessment, which may increase its accessibility beyond traditional lab-based measures.

INFLAMMATION

Inflammation is a common and well known factor influencing iron assessment and is described as a biological confounding factor. Ferritin, commonly used to assess iron stores, is an acute-phase protein that increases in response to inflammation making it less useful as a biomarker. Distinguishing anemia of inflammation from true iron deficiency anemia remains a gap in knowledge, and MRI offers the potential to assess iron content in multiple tissues including in the liver, pancreas and heart [3]. This approach was noted as having the potential to better identify and treat people with anemia of inflammation, typically characterized with lower serum iron and transferrin saturation and higher ferritin levels compared to people with anemia of inflammation and iron deficiency anemia.

Recent research demonstrates heterogeneity in associations between iron status and inflammation suggesting that the mechanism explaining iron levels and inflammatory markers is not consistent across observational studies. One study using cluster analysis to categorize the inflammatory content of diet derived from dietary records, body size, and biomarkers [4] found no association between inflammatory status and iron status biomarkers but did show an inverse association between total iron-binding capacity and inflammatory diet content. When focusing on associations between inflammatory biomarkers and anemia status in an older sample of adults $\geq$ 65 years [5], inflammatory biomarkers such as tumor necrosis factor (TNF)-alpha, hepcidin, and interleukin (IL)-1-beta were higher in the group defined as having anemia (based on hemoglobin levels), but not the C-reactive protein (CRP) biomarker. When examining another type of inflammation biomarker based on cell counts of neutrophil, lymphocytes, and platelets, authors of a study [6] based on a US population found an inverse relationship with serum ferritin, which is contrary to a positive relationship commonly found with inflammatory markers such as CRP. The authors hypothesized that ferritin may be positively associated with cell damage-based biomarkers such as CRP, but not cell counts in the systemic immune-inflammation composite marker used in their study, suggesting inconsistent associations between iron assessment and biomarkers for inflammation. Last, in another study of indigenous lactating mothers in Panama of childbearing age, there was no association between inflammation indicators and anemia, but there was a positive association with hepcidin and ferritin alongside an inverse association with serum iron [7]. These studies include populations from different countries as well as different age groups, which, alongside the observational character of all these studies, could contribute to the variation in results.

IRON ASSESSMENT IN WOMEN

Research focusing on factors such as heavy menstrual bleeding [8] or weight change among young women with overweight [9▪] suggest these are subgroups that are important candidates for iron assessment. In a group of adolescents presenting to an emergency department for heavy menstrual bleeding ($n = 258$), a large proportion of those with an iron study ($n = 23$) had iron deficiency (78%) and anemia (92%). These findings suggest the need for increasing iron status assessments in adolescents with heavy menstrual bleeding, and they support existing evidence that heavy bleeding is a predictor of lower iron stores. A study reporting results from a randomized weight loss intervention [9▪] hypothesized weight loss would result in lower inflammation and subsequently alter iron levels. As expected, following the intervention, weight loss was associated with lower inflammatory markers, including CRP and hepcidin, as well as higher levels for hemoglobin, ferritin, and serum iron. Interestingly, participants in the weight loss intervention group consumed less iron but had higher iron levels than the control group, suggesting a mechanism unrelated to iron intake and perhaps relating to inflammation.

Other studies assessed multiple predictors of iron status in women during their childbearing years [7] and/or menopause [10]. In an observational study of lactating indigenous women in Panama [7] discussed above, CRP, an inflammatory marker, was positively associated with hepcidin and ferritin and inversely associated with serum iron. In the study of survey-based self-reported responses from participants, recent blood donation was a factor

having the strongest association with iron outcomes for both pre- and postmenopausal women [10].

Altogether, these studies suggest that the association between inflammation and iron varies across populations. At the same time, these studies reinforce the existing literature stressing the consideration of concurrent inflammation when assessing iron content in people. Furthermore, defining alternative thresholds for conditions such as anemia and iron overload also requires additional research to better understand the role and mechanism underlying associations between inflammation and iron status in subgroups.

NON-SERUM BASED ASSESSMENT METHODS

Alternate methods to iron assessment through classic serum iron biomarkers from standard assays such as serum iron, ferritin, and transferrin [2] can be useful and fill gaps in access to standard tests and tissue-specific detection. A new method [11], measuring concentrations of apotransferrin in Ethylenediaminetetraacetic acid (EDTA)-treated plasma, is proposed as a proxy to unsaturated iron-binding capacity (UIBC) and has fewer requirements for equipment making it suitable for low resource settings. Similarly, there was a validation of VitMin Lab s-ELISA assays [12] for iron levels such as ferritin compared to traditional iron measures from NHANES. More than 88% of the ferritin samples in this study were within 30% of the referent and comparable prevalence estimates for abnormal measures based on ferritin, making it a candidate for use in low-resource settings. When compared to the s-ELISA assay, another study noted that the 7-plex assay [13], another candidate assay to assess ferritin in low-resource settings, had ferritin measures that were correlated but consistently higher than the referent lessening its diagnostic value.

Iron levels measured in nails present a less invasive and easier to store resource, but a recent study comparing common serum iron measures to iron levels in nails [14] did not find substantive associations between these two biomarkers. When measuring iron levels, nails may not offer a good proxy to serum-based measures. A different study [15] evaluating a device to assess ferritin in blood using capillary blood samples, validated and compared the new method to the traditional method using venous serum and found a strong correlation ($r^2 = 0.86$) between the two. When using this method to diagnose iron deficiency using a WHO cutoff, they found a sensitivity of 0.90 and specificity of 0.96. This method has advantages in terms of cost and accessibility compared to more time-intensive and invasive methods. Another recent report detailed a prototype for real-time ferritin detection using a novel biosensor [16]. Pending future development and scaling of this method, it has potential to provide iron assessment in point-of-care settings requiring lower costs of detection. These new methods offer progress towards increasing iron assessment capabilities in low-resource settings.

Artificial intelligence may have a potential role in the assessment of iron status, especially in its ability to reach larger groups of people, but this nascent method requires more development [17]. The possibility to assess iron deficiency anemia with a smartphone app and artificial intelligence was explored by evaluating images of conjunctiva [18]. One of their approaches demonstrated a sensitivity of 79% and specificity of 74%. Another study using machine learning methods to classify four different types of anemia (beta thalassemia trait, hemoglobin E, iron deficiency anemia, and specific combinations of the three prior types) conditional on a person being diagnosed with anemia [19] demonstrated a sensitivity exceeding 98%. With larger and more diverse training sets these methods could also be useful in low-resource settings with less access to traditional serum-based assessment methods.

MRI methods, used to assess iron status and more likely to be available in high-resource settings, have capabilities superior to common serum-based assays in detecting iron content in tissues. As discussed above, a study assessing iron retention in patients with anemia of inflammation, confirmed findings from animal models of iron retention in macrophages [3]. These findings point towards improved biomarker thresholds to assess iron deficiency in patients with inflammatory conditions. In a group of thalassemic patients, an MRI method evaluated iron overload in heart, liver and pancreas tissues to measure chelation therapy response [20]. Interestingly, in this study, the authors found that serum ferritin decreased before iron overload in the liver suggesting that decreasing serum ferritin signaled improvement in iron overload in other tissues. Another recent study also found a positive association between liver and cardiac overload and serum ferritin measures [21]. However, while useful in measuring iron overload, serum ferritin cannot provide tissue-specific iron levels.

When validating MRI technology using in-vitro, in-vivo, and ex-vivo means to assess the molecular state of iron in the brain, a recent study suggests MRI is also useful as a noninvasive means to assess iron status in brain tissue [22▪▪]. Among some of their findings, the authors were able to detect patterns of iron homeostasis in brain tumors of meningioma

patients and suggested the new method can be used in noninvasive research into the human brain. Last, a recent report outlined best practices for quantifying liver iron content using MRI, based on expert panel recommendations and a literature review from the last two decades [23]. These guidelines can provide better alignment of future research methods focusing on tissue-specific iron level assessment. Overall, these recent papers demonstrate the expanding scope of tissue-specific detection of iron levels that can provide a better understanding of iron metabolism.

ABNORMAL LEVELS: IRON OVERLOAD AND DEFICIENCY

Iron deficiency and iron overload are prevalent health outcomes, and their assessment remains a source of potential misclassification with existing assessment methods. Research is active given the lack of consensus on the definition of these outcomes.

MRI technology can provide an alternate, and less invasive, measure for iron overload in the liver [23,24]. Iron accumulation in the body due to metabolic hyperferritinemia is the primary topic of a comprehensive literature review, which concludes with an updated definition and staging system with cutoffs of liver iron concentrations assessed with MRI [25]. This characterization of iron accumulation is meant to advance research and guide decision-making for metabolic hyperferritinemia. Another group introduced and validated the concept of a liver iron index as measured through MRI instead of biopsy [24], finding this measure to be effective in distinguishing between major and minor iron overload in patients with hyperferritinemia.

Iron deficiency is an adverse health outcome with biomarker cutoffs that depend on the health status of an individual, and, if adhering to general iron thresholds for diagnosis, may result in failure to identify people with low iron stores who could receive therapy to improve their health status. Outdated guidelines for the assessment of iron deficiency are outlined in a recent essay on iron deficiency in the United States [26], prompting a call for improved and systematic recommendations of laboratory results. Similarly, in people with inflammatory conditions, iron deficiency does not have a consensus on its assessment with common parameters such as ferritin. Among patients with inflammatory conditions around half may be deficient in iron, noted in a recent review [27] that advocates for assessment of iron deficiency in all people with chronic inflammatory diseases such as chronic heart failure, chronic kidney disease, inflammatory bowel disease, and cancer.

A recent review of the potential for hepcidin as a new biomarker for anemia [28] discusses the potential to fill existing gaps in iron deficiency assessment. Given the role of hepcidin in iron homeostasis, it may fulfill the need for alternative biomarkers when assessing iron status in certain groups, including those experiencing chronic disease driven inflammatory anemia and infants with iron deficiency. Using patient records, a recent study evaluated hepcidin as a means to differentiate between iron-refractory iron-deficiency anemia and anemia associated with gastrointestinal causes [29]. Using a combination of hepcidin and oral iron absorption tests, the authors found unique associations suggesting different causal mechanisms such as menstrual blood loss, genetic conditions, and malabsorption. Incorporating hepcidin as a biomarker into existing methods may improve assessment of types of iron deficiency in people with inflammation.

CONCLUSION

This review of recent literature focusing on iron assessment addressed some common themes including influencing factors, new methods, new biomarkers, and existing problems in iron assessment for conditions such as iron deficiency and overload. Some of these findings show gaps in existing iron assessment approaches: iron deficiency in people with inflammatory conditions being one example. More sophisticated screening methods such as MRIs are in a position to advance the understanding of areas such as tissue-specific iron metabolism, which can better inform interventions and noninvasive research. The mechanisms by which iron levels change are better defined than the messages regarding how and in who to assess iron levels, which is motivating some of the discussed research. In summary, this review highlights recent research on iron assessment that focuses on increasing access, updating standards, and developing new methods to improve accuracy of diagnoses and increase interventions to improve health.

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Conflicts of interest

Ann Von Holle has no conflict of interest to report.

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- ■ of special interest
- ■■ of outstanding interest

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